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Loss of the N-myc Oncogene in a Patient With a Small Interstitial Deletion of the Short Arm of Chromosome 2

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To our knowledge, only four previous cases of distal chromosome 2p deletions exist in the literature. We present a patient with minor facial anomalies who had a distal interstitial deletion of the short arm of chromosome 2, del(2)(p24.2p25.1). This patient had many features seen in other patients with distal 2p deletion including short stature, "rectangular" facies, microcephaly, hypotonia, and mental retardation. This patient also has sensorineural hearing loss which has been described in one other patient with a similar deletion. The N-myc oncogene has been mapped to 2p24. By fluorescence in situ hybridization using a cDNA probe for the N-myc oncogene, this patient was found to have a deletion of the N-myc oncogene. This confirms the previous map location for N-myc.

KEY WORDS: chromosome deletion, mental retardation, dysmorphic syndrome, fluorescent in situ hybridization

INTRODUCTION

Deletions of the distal portion of 2p are rare, with only 4 cases of deletions involving regions from 2p23 to 2pter having been reported [Emanuel et al., 1979; Penchaszadeh et al., 1987; Neidich et al., 1987] (the report by Neidich et al. [1987] is a follow up of case two first reported by Emanuel et al. [1979]); whereas one case involved a terminal deletion of chromosome 2p24 [Francis et al., 1990]. We report on a boy with minor anomalies with the smallest deletion of this region to date. High-resolution cytogenetic analysis suggests that the breakpoints on the short arm of chromosome 2 are p24.2 and p25.1.

The N-myc oncogene has been mapped to 2p24 [Garson et al., 1987]. Utilizing fluorescence in situ hybridization (FISH) and a DNA probe for the N-myc oncogene, we found our patient to be hemizygous for that gene, thus confirming the gene map assignment of N-myc.

CLINICAL REPORT

The patient was a 10-month-old African American male who was evaluated initially in an outreach genetics clinic because of minor facial anomalies and developmental delay. He was the 3,040 g product of a full term normal vaginal delivery to a 22-year-old gravida 3 para 2 woman and her non-consanguineous 22-year-old partner. The pregnancy was uncomplicated, and there were no illnesses, exposures, use of alcohol, tobacco, or drugs. No prenatal testing was performed and maternal weight gain was 18 kg. The patient remained hospitalized for 9 days for management of hyperbilirubinemia with phototherapy. The early medical history was otherwise unremarkable.

At 10 months, the boy had an unusual facial appearance and was hypotonic with global developmental delays. Weight was 7.50 kg (<5th centile; 50th centile for 5 months), length was 68.8 cm (<5th centile), and occipitofrontal circumference (OFC) was 42.5 cm (<5th centile; 50th centile for 5 months). The skin was clear. The head was microbrachycephalic with a patent anterior fontanelle, mild synophrys, and arching of the eyebrows bilaterally. The face was "rectangular." Palpebral fissures were horizontal with an inner canthal distance of 3.0 cm (97th centile) and an outer canthal distance of 8.2 cm (97th centile). The ears were low set, mildly cupped with over folded superior helices. The palate had a normal contour and was intact; the dentition was normal. There was oromotor hypotonia with persistent drooling. The neck was supple with a non-fixed left

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torticollis and could easily be brought to neutral. There were no cardiac murmurs. The abdomen was soft and non-tender with a prominent diastasis recti and no organomegaly. Genitalia were Tanner stage 1 with both testes descended. There was fifth finger clinodactyly bilaterally. Neurologic examination revealed orofacial, appendicular, and axial hypotonia.

Developmental examination was performed on the patient at age 11 months utilizing the Bayley Scales of Infant Development [Bayley, 1969] and the Gesell Developmental Schedules [Knobloch et al., 1980]. His gross motor function was at a 5 month level, fine motor function at a 6 month level, and cognitive skills were at 6.2 months with a Mental Development Index (MDI) of 50. Receptive language and expressive language skills were at 5 months, and personal social skills were at 6 months. In addition to the above findings, the patient demonstrated asymmetric response to auditory stimuli. Brainstem auditory evoked response testing performed at 12 months demonstrated severe to profound hearing loss in the right ear with mild hearing loss in the left ear.

Follow-up examination at age 24 months revealed that the patient was walking at 12 months, and began speaking single words at 22 months. Weight was 11.14 kg (10th–5th centile), length was 78.7 cm (<5th centile; 50th centile for 14 months), and OFC was 44.5 cm (<5th centile; 50th centile for 7 months). He was also noted to have tapering of the digits with brachyclinodactyly of both fifth fingers and brachydactyly of the toes. There continued to be mild hypotonia (Figs. 1, 2).

Family history revealed that the patient had two healthy older sisters, both parents were in good health, and there was no history of mental retardation, learning disability, deafness, stillbirths, or recurrent pregnancy loss.

CYTOGENETIC AND FISH ANALYSIS

High-resolution chromosome analysis at approximately the 850 band level demonstrated an interstitial deletion of part of band 2p24. The likely breakpoints are at p24.2 and p25.1 resulting in the absence of band p24.3 (Fig. 2). However, owing to the small size of the deletion, the alternative interpretation, which cannot be completely excluded, is that band 2p24.1 is deleted with breakpoints at bands p23.3 and p24.2. Both parents had normal chromosomes.

FISH analysis was performed on a fibroblast cell line established from a skin biopsy. Cells from this line were simultaneously hybridized with a Spectrum Aqua™

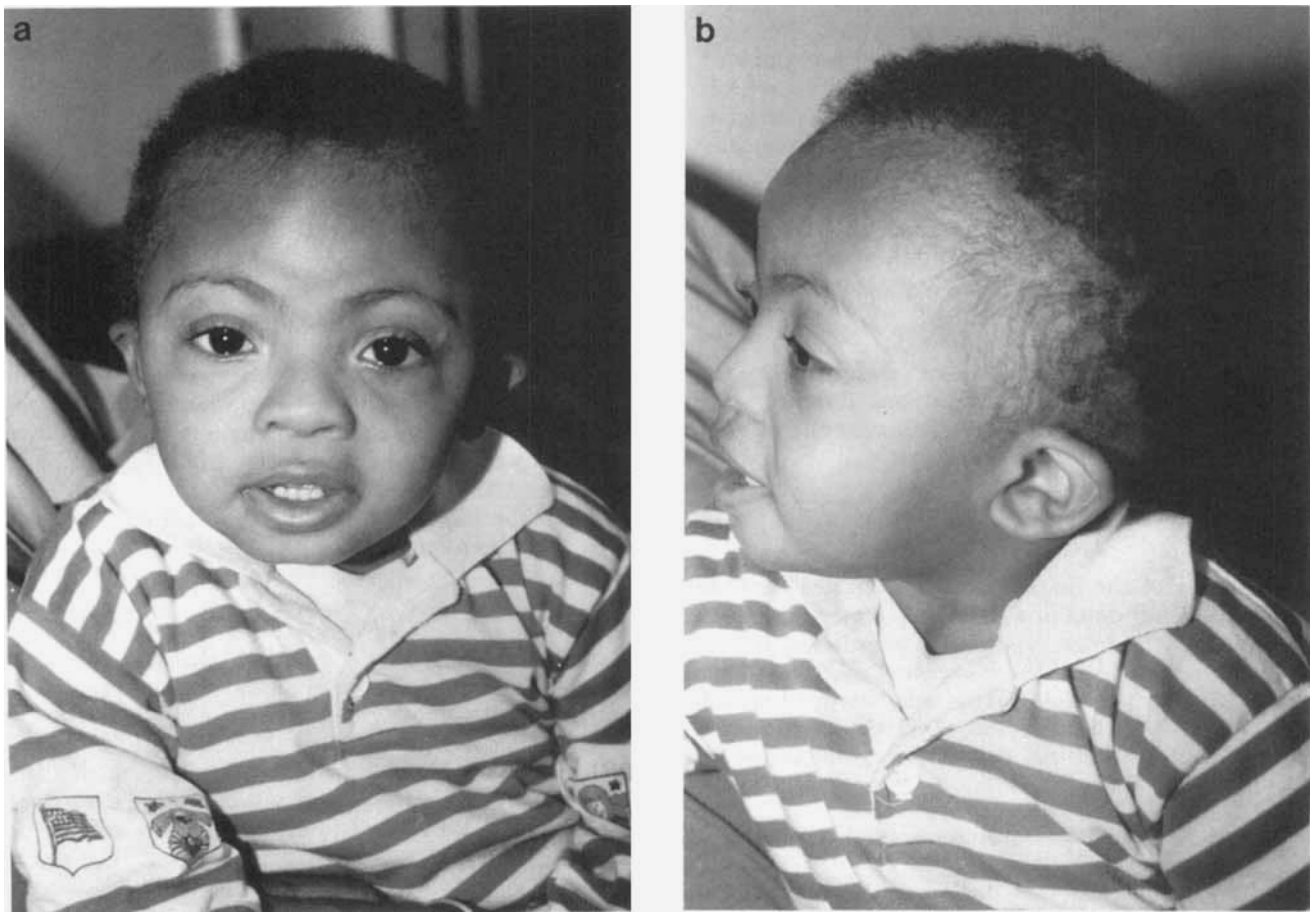

Fig. 1. **a,b**: The patient at age 2 years.

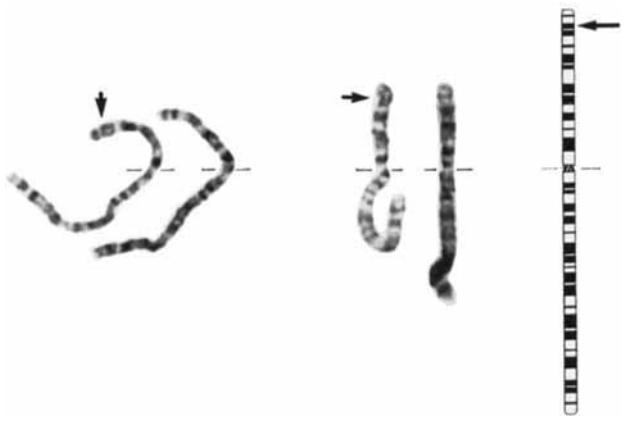

Fig. 2. Two partial GTG-banded karyotypes of chromosome 2. The normal homologue is on the left of each pair and the arrow points to band 2p24.3 which is absent in the deleted homologue (right). The normal idiogram of chromosome 2 is on the far right.

WCP™ chromosome painting probe and Spectrum Orange™ N-myc oncogene probe. Analysis of the metaphase cells showed that the N-myc probe hybridized to one of the homologues of chromosome 2, but not to the other (Fig. 3). This indicates that the cytogenetic deletion includes the N-myc locus.

DISCUSSION

Although no specific phenotype exists for distal interstitial deletions of chromosome 2p, this patient has several findings described in previously reported patients with 2p deletions, including microcephaly, "rectangular" face, apparently low-set ears, fifth finger clinodactyly, hypotonia, and developmental delay (Table I) [Emmanuel et al., 1979; Penchaszadeh et al., 1987; Neidich et al., 1987; Francis et al., 1990]. The patient reported by Neidich et al. [1987] is a same one originally reported by Emmanuel et al. [1979]. Micrognathia, high forehead, and failure to thrive, each seen in three of the previously reported patients, were absent in our patient. A unique finding in our patient is hypertelorism, although, in two of the previous cases, measurements of intraorbital distances were not reported [Emmanuel et al., 1979; Francis et al., 1990]. Our patient had sensorineural hearing loss with profound loss in the right ear and mild loss in the left ear. The patient reported by Francis et al. [1990] also had profound hearing loss, suggesting that all patients with chromosome 2p abnormalities should have audiological evaluations. Recent studies of N-myc expression in the embryonic cochlea of the mouse has demonstrated a significant level of N-myc transcript in the otic placode during late embryogenesis. This suggests a possible relationship between N-myc expression and cell differentiation in the inner ear [Romand et al., 1994].

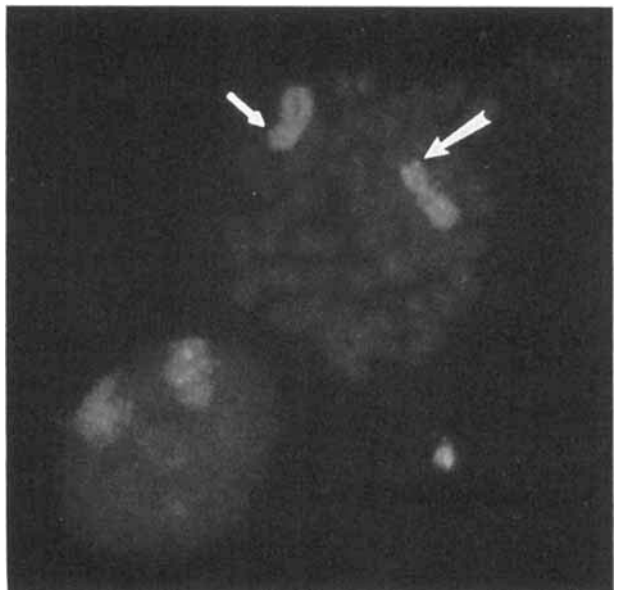

Fig. 3. Fibroblast metaphase hybridized with Spectrum Aqua™ WCP™ chromosome 2 painting probe and Spectrum Orange™ N-myc probe. The large arrow indicates the normal chromosome 2 with fluorescent N-myc signals. The small arrow identifies the abnormal deleted homologue lacking N-myc hybridization.

The N-myc oncogene was mapped to 2p24 utilizing biotin labeled DNA probes hybridized to normal human lymphocytes [Garson et al., 1987]. By using FISH and cells from our patient who has a partial deletion of band 2p24 from one of his chromosome 2 homologues, we have confirmed that N-myc maps to 2p24 by absence of hybridization of the N-myc DNA probe to the deleted homologue.

Myc proteins are site-specific DNA-binding proteins which belong to a group of transcription factors that control such diverse processes as myogenesis, neurogenesis, and sex determination [Garrell and Campuzano, 1991; Moens et al., 1993]. N-myc is expressed predominantly in the embryo in undifferentiated subsets of cells in the central and peripheral nervous system, lung, kidney, and eye [Mugrauer et al., 1988; Hirning et al., 1991; Zimmerman et al., 1986]. In mice with null mutations of N-myc there is a reduced number of mesonephric tubules, the genital ridge is hypoplastic, and there is a defect in the development of the stomach and intestine [Katoh et al., 1991; Mugrauer et al., 1988; Hirning et al., 1991; Stanton et al., 1992; Moens et al., 1993]. Reduced levels of N-myc protein in mouse embryos results in thinning of the subepicardial compact layer of the myocardium by 12.5 days of development. It is of interest that the phenotypes of patients with 2p24 deletions, and presumed N-myc deletions are not consistent with these findings in mice. Renal anomalies have not been appreciated. There has been no report of cryptorchidism or ambiguous genitalia in the four males reported. Congenital heart defects have been seen in two patients [Penchaszadeh, 1987; Francis, 1990]. Neurologic abnormalities including hypotonia, seizures, and mental retardation have been noted in all patients with 2p deletions; however, it is as likely that this is caused by having a chromosome deletion with loss of several genes and imbalance of genetic material and as being caused solely by a deletion of the N-myc gene.

TABLE I. Clinical Findings in del(2)(p24)*

Manifestation	Present case	Emmanuel et al., 1979 Case 1	Penchaszadeh et al., 1987	Neidich et al., 1987	Francis et al., 1990
Sex	Male	Female	Male	Male	Male
Birth weight (g)	2,730	3,500	2,950	2,800	2,300
Microcephaly	+	+	+	+	+
Brachycephaly	+	−	−	−	+
Prominent occiput	−	−	+	+	−
Prominent metopic suture	−	−	+	+	−
Rectangular face	+	+	+	+	+
Ptosis	−	−	−	+	−
High forehead	−	+	+	+	−
Arched eyebrows	+	−	−	+	−
Hypertelorism	+	NA	−	−	NA
Strabismus	−	+	+	−	−
Down slanting palpebral fissures	−	+	−	+	−
Apparently low-set ears	+	+	−	+	−
Bow shaped mouth	−	+	+	+	+
High arched palate	−	−	+	+	+
Micrognathia	−	+	+	−	+
Fifth finger clinodactyly	+	+	+	+	+
Congenital heart defect	−	−	+	−	+
Hearing loss	+	−	−	−	+
Hypotonia	+	+	+	−	+
Failure to thrive	−	+	+	+	−
Seizures	−	−	+	+	+
Developmental delay/mental retardation	+	+	+	+	+

*+, present; −, absent; NA, information not available.

Although the patient is hemizygous for the N-myc oncogene, it is not possible to predict what the future health consequences of this event will be for this individual; although, no malignancies have been reported in the other cases with 2p24 deletions [Emmanuel et al., 1979; Penchaszadeh et al., 1987; Neidich et al., 1987; Francis et al., 1990]. The N-myc gene has been shown to be amplified 25- to 700-fold in eight of nine human neuroblastoma cell lines [Kohl et al., 1983]. This mechanism of carcinogenesis is dissimilar from that which is seen in other deletion syndromes associated with malignancies, such as retinoblastoma, for which the germline deletion of the RB1 gene is the first of two events which must occur for carcinogenesis, as initially postulated by Knudson [1971].

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